

IN THE SPECIFICATION

Please amend the paragraph at page 23, lines 13-26 as follows:

As used herein, the SLC gene and SLC protein includes the murine and human SLC genes and proteins specifically described herein, as well as biologically active structurally and/or functionally similar variants or analog of the foregoing. SLC peptide analogs generally share at least about 50%, 60%, 70%, 80%, 90% or more amino acid homology (using BLAST criteria). For example, % identity values may be generated by WU-BLAST-2 (Altschul et al., 1996, Methods in Enzymology 266:460-480; <http://blast.wustl.edu/blast/README.html>). SLC nucleotide analogs preferably share 50%, 60%, 70%, 80%, 90% or more nucleic acid homology (using BLAST criteria). In some embodiments, however, lower homology is preferred so as to select preferred residues in view of species-specific codon preferences and/or optimal peptide epitopes tailored to a particular target population, as is appreciated by those skilled in the art. Fusion proteins that combine parts of different SLC proteins or fragments thereof, as well as fusion proteins of a SLC protein and a heterologous polypeptide are also included. Such SLC proteins are collectively referred to as the SLC-related proteins, the proteins of the invention, or SLC.

Please amend the paragraph bridging pages 37-38 of the specification as follows:

Plant alkaloids such as ~~taxol~~ TAXOL are also contemplated for use in certain aspects of the present invention. ~~Taxol~~ TAXOL is an experimental antimitotic agent, isolated from the bark of the ash tree, *Taxus brevifolia*. It binds to tubulin (at a site distinct from that used by the vinca alkaloids) and promotes the assembly of microtubules. ~~Taxol~~ TAXOL is currently being evaluated clinically; it has activity against malignant melanoma and carcinoma of the ovary. Maximal doses are 30 mg/m² per day for 5 days or 210 to 250 mg/m² given once every 3 weeks. Of course, all of these dosages are exemplary, and any dosage in-between these points is also expected to be of use in the invention.

Please amend the paragraphs at page 60, line 34 to page 64, line 15 as follows:

Two weakly immunogenic lung cancers, line 1 alveolar carcinoma (L1C2, H-2d) and Lewis lung carcinoma (3LL, H-2b), were utilized for assessment of antitumor responses in vivo. The cells were routinely cultured as monolayers in 25-cm³ tissue culture flasks containing RPMI 1640 (~~Irvin~~

~~Scientific~~ IRVINE SCIENTIFIC, Santa Ana, CA) supplemented with 10% FBS (~~Gemini Bioproducts~~ GEMINI BIOPRODUCTS, Calabasas, CA), penicillin (100 U/ml), streptomycin (0.1 mg/ml), 2 mM glutamine (~~JRH Biosciences~~ JRH BIOSCIENCES, Lenexa, KS) and maintained at 37°C in a humidified atmosphere containing 5% CO₂ in air. The cell lines were Mycoplasma free, and cells were utilized up to the tenth passage before thawing frozen stock cells from liquid N₂. For tumorigenesis experiments, 105 3LL or L1C2 tumor cells were inoculated by s.c. injection in the right suprascapular area of C57BL/6 or BALB/c mice, and tumor volume was monitored three times per week. Five-day-old established tumors were treated with intratumoral injection of 0.5 µg of murine recombinant SLC or PBS diluent (~~Peppo Tech~~ PEPRO TECH, Rocky Hill, NJ) administered three times per week for 2 weeks. The endotoxin level reported by the manufacturer was <0.1 ng/µg (1 EU/µg) of SLC. The amount of SLC (0.5 µg) used for injection was determined by the in vitro biological activity data provided by the manufacturer. Maximal chemotactic activity of SLC for total murine T cells was 100 ng/ml. For in vivo evaluation of SLC-mediated antitumor properties, we utilized 5-fold more than this amount for each intratumoral injection. Tumorigenesis experiments were also performed in which equivalent amounts of murine serum albumin were utilized (~~Sigma~~ SIGMA, St. Louis, MO) as an irrelevant protein for control injections. Experiments were also performed in which the SLC was administered at the time of tumor inoculation. To determine the importance of the immune system in mediating antitumor responses after SLC administration, tumorigenesis experiments were conducted in SCID beige CB17 mice. SLC was administered s.c. at the time of tumor inoculation and then three times per week. CD4 and CD8 knockout mice were utilized to determine the contribution of CD4 and CD8 cells in tumor eradication. Two bisecting diameters of each tumor were measured with calipers. The volume was calculated using the formula $(0.4)(ab^2)$, with a as the larger diameter and b as the smaller diameter.

2. Cytokine determination from tumor nodules, lymph nodes, and spleens

The cytokine profiles in tumors, lymph nodes, and spleens were determined in both SLC and diluent-treated mice as previously described (Sharma et al., J. Immunol. 163:5020). Non necrotic tumors were harvested, cut into small pieces, and passed through a sieve (~~Belleo Glass~~ BELLCO GLASS, Vineland, NJ). Tumor-draining lymph nodes and spleens were harvested from SLC-treated tumor-bearing, control tumor-bearing, and normal control mice. Lymph nodes and spleens were

teased apart, RBC depleted with double-distilled H₂O, and brought to tonicity with 1x PBS. Tumor nodules were evaluated for the production of IL-10, IL-12, GM-CSF, IFN- γ , TGF- β , vascular endothelial growth factor (VEGF), monokine induced by IFN- γ (MIG), and IP-10 by ELISA and PGE₂ by enzyme immunoassay (EIA) in the supernatants after an overnight culture. Tumor-derived cytokine and PGE₂ concentrations were corrected for total protein by Bradford assay (~~Sigma~~ SIGMA, St. Louis, MO). For cytokine determinations after secondary stimulation with irradiated tumor cells (5×10^6 cells/ml), splenic or lymph node-derived lymphocytes were cocultured with irradiated 3LL (105 cells/ml) at a ratio of 50:1 in a total volume of 5 ml. After an overnight culture, supernatants were harvested and GM-CSF, IFN- γ , IL-12, and IL-10 determined by ELISA.

3. Cytokine ELISA

Cytokine protein concentrations from tumor nodules, lymph nodes and spleens were determined by ELISA as previously described (Huang et al., Cancer Res. 58:1208). Briefly, 96-well ~~Costar~~ COSTAR (Cambridge, MA) plates were coated overnight with 4 μ g/ml of the appropriate anti-mouse mAb to the cytokine being measured. The wells of the plate were blocked with 10% fetal bovine serum (~~Gemini-Bioproducts~~ GEMINI BIOPRODUCTS) in PBS for 30 min. The plate was then incubated with the Ag for 1 h, and excess Ag was washed off with PBS-Tween. The plate was incubated with 2 μ g/ml biotinylated mAb to the appropriate cytokine (~~PharMingen~~ PHARMINGEN, San Diego, CA) for 30 min, and excess Ab was washed off with PBS-Tween. The plates were incubated with avidin peroxidase, and after incubation in OPD substrate to the desired extinction, the subsequent change in color was read at 490 nm with a Microplate Reader (~~Molecular Dynamics~~ MOLECULAR DYNAMICS, Sunnyvale, CA). The recombinant cytokines used as standards in the assay were obtained from ~~PharMingen~~ PHARMINGEN. IL-12 (~~Biosource~~ BIOSOURCE) and VEGF (~~Oncogene Research Products~~ ONCOGENE RESEARCH PRODUCTS, Cambridge, MA) were determined by kits according to the manufacturer's instructions. MIG and IP-10 were quantified by a modification of a double ligand method as previously described (Standiford et al., J. Clin. Invest. 86:1945). The MIG and IP-10 Abs and

protein were from R&D (Minneapolis, MN). The sensitivities of the IL-10, GM-CSF, IFN- γ , TGF- β , MIG, and IP-10 ELISA were 15 pg/ml. For IL-12 and VEGF, the sensitivities were 5 pg/ml.

4. PGE2 EIA

PGE2 concentrations were determined using a kit from ~~Cayman Chemical~~ CAYMAN CHEMICAL (Ann Arbor, MI) according to the manufacturer's instructions as previously described (Huang et al., Cancer Res. 58:1208). The EIA plates were read by a ~~Molecular Dynamics~~ MOLECULAR DYNAMICS Microplate Reader.

5. Cytolytic experiments

Cytolytic activity was assessed as previously described (Sharma et al., J. Immunol. 163:5020). To quantify tumor cytolysis after a secondary stimulation with irradiated tumor cells, lymph node-derived lymphocytes (5×10^6 cells/ml) from SLC-treated and diluent tumor-bearing mice were cultured with irradiated 3LL (10^5 cells/ml) tumors at a ratio of 50:1 in a total volume of 5 ml. After a 5-day culture, the lytic capacity of lymph node-derived lymphocytes were determined against chromium-labeled (^{51}Cr , ~~Amersham~~ AMERSHAM Arlington, Heights, IL; sp. act. 250–500 mCi/mg) 3LL targets at varying E:T ratios for 4 h in 96-well plates. Spontaneous release and maximum release with 5% ~~Triton~~ Triton X also were assessed. After the 4-h incubation, supernatants were removed and activity was determined with a gamma counter (~~Beckman~~ BECKMAN, Fullerton, CA). The percent specific lysis was calculated by the formula: % lysis = $100 \times (\text{experimental cpm} - \text{spontaneous release}) / (\text{maximum release} - \text{spontaneous release})$.

6. Flow cytometry

For flow cytometric experiments, two or three fluorochromes (PE, FITC, and Tri-color) (~~PharMingen~~ PHARMINGEN) were used to gate on the CD3 T lymphocyte population of tumor nodule single-cell suspensions. DCs were defined as the CD11c and DEC 205 bright populations within tumor nodules and lymph nodes. Cells were identified as lymphocytes or DC by gating based on forward and side scatter profiles. Flow cytometric analyses were performed on a FACScan flow cytometer (~~Becton Dickinson~~ BECTON DICKINSON, San Jose, CA) in the University of

California, Los Angeles, Jonsson Cancer Center Flow Cytometry Core Facility. Between 5,000 and 15,000 gated events were collected and analyzed using ~~Cell-Quest~~ CELL QUEST software (~~Becton Dickinson~~ BECTON DICKINSON).

7. Intracellular cytokine analysis

T lymphocytes from single-cell suspensions of tumor nodules and lymph nodes of SLC-treated and diluent-treated 3LL tumor-bearing mice were depleted of RBC with distilled, deionized H₂O and were evaluated for the presence of intracytoplasmic GM-CSF and IFN- γ . Cell suspensions were treated with the protein transport inhibitor kit GolgiPlug (~~PharMingen~~ PHARMINGEN) according to the manufacturer's instructions. Cells were harvested and washed twice in 2% FBS-PBS. Cells (5×10^5) cells were resuspended in 200 μ l of 2% FBS-PBS with 0.5 μ g FITC-conjugated mAb specific for cell surface Ags CD3, CD4, and CD8 for 30 min at 4°C. After two washes in 2% FBS-PBS, cells were fixed, permeabilized, and washed using the Cytofix/Cytoperm Kit (~~PharMingen~~ PHARMINGEN) following the manufacturer's protocol. The cell pellet was resuspended in 100 μ l Perm/Wash solution and stained with 0.25 μ g PE-conjugated anti-GM-CSF and anti- IFN- γ mAb for intracellular staining. Cells were incubated at room temperature in the dark for 30 min, washed twice, resuspended in 300 μ l PBS, 2% paraformaldehyde solution, and analyzed by flow cytometry.

Please amend the paragraphs at page 68, line 28 to page 72, line 25 as follows:

Clara cell lung tumor cells (CC-10 Tag and H-2q) were derived from freshly excised lung tumors that were propagated in RPMI 1640 (~~Irvine Scientific~~ IRVINE SCIENTIFIC, Santa Ana, CA) supplemented with 10% FBS (~~GeminiBioproducts~~ GEMINI BIOPRODUCTS, Calabasas, CA), penicillin (100 units/ml), streptomycin (0.1mg/ml), and 2 mM of glutamine (~~JRH Biosciences~~ JRH BIOSCIENCES, Lenexa, KS) and maintained at 37°C in humidified atmosphere containing 5% CO₂ in air. After two *in vivo* passages, CC-10 TAg tumor clones were isolated. The cell lines were *Mycoplasma* free, and cells were used up to the tenth passage before thawing frozen stock cells from liquid N₂.

2. CC10TAg Mice.

The transgenic CC-10 TAg mice, in which the SV40large TAg is expressed under control of the murine Clara cell-specific promoter, were used in these studies (Magdaleno et al., Cell Growth Differ., 8: 145–155, 1997). All of the mice expressing the transgene developed diffuse bilateral bronchoalveolar carcinoma. Tumor was evident bilaterally by microscopic examination as early as 4 weeks of age. After 3 months of age, the bronchoalveolar pattern of tumor growth coalesced to form multiple bilateral tumor nodules. The CC-10 TAg transgenic mice had an average life span of 4 months. Extrathoracic metastases were not noted. Breeding pairs for these mice were generously provided by Francesco J. DeMayo (Baylor College of Medicine, Houston, TX). Transgenic mice were bred at the West Los Angeles Veteran Affairs vivarium and maintained in the animal research facility. Before each experiment using the CC-10 TAg transgenic mice, presence of the transgene was confirmed by PCR of mouse tail biopsies. The 5' primer sequence was SM19-TAG: 5'-TGGACCTTCTAGGTCTTGAAAGG-3' (SEQ ID NO: 3), and the 3' primer sequence was SM36-TAG: 5'-AGGCATTCCACCACTGCTCCCAT-3' (SEQ ID NO: 4). The size of the resulting PCR fragment is 650 bp. DNA (1 µg) was amplified in a total volume of 50 µl, which contained 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 200 µM each deoxynucleotidetriphosphates, 0.1 µM primers, 2.5 mM MgCl₂, and 2.5 units of Taq polymerase. PCR was performed in a ~~Perkin-Elmer~~ PERKIN-ELMER DNA thermal cycler (Norwalk, CT). The amplification profile for the SV40 transgene consisted of 40 cycles, with the first cycle denaturation at 94°C for 3 min, annealing at 58°C for 1 min, and extension at 72°C for 1 min, followed by 39 cycles with denaturation at 94°C for 1 min, and the same annealing and extension conditions. The extension step for the last cycle was 10 min. After amplification, the products were visualized against molecular weight standards on a 1.5% agarose gel stained with ethidium bromide. All of the experiments used pathogen-free CC-10 TAg transgenic mice beginning at 4–5 week of age.

3. The SLC Therapeutic Model in CC-10 TAg Mice.

CC-10 TAg transgenic mice were injected in the axillary node region with murine recombinant SLC (0.5 µg/injection; ~~Peppo-Tech~~ PEPRO TECH, Rocky Hill, NJ) or normal saline diluent, which contained equivalent amounts of murine serum albumin (~~Sigma-Chemical~~ SIGMA CHEMICAL Co.,

St. Louis, MO) as an irrelevant protein for control injections. Beginning at 4–5 weeks of age, SLC or control injections were administered three times per week for 8 weeks. The endotoxin level reported by the manufacturer was <0.1 ng/ μ g (1 endotoxin unit/ μ g) of SLC. The dose of SLC (0.5 μ g/injection) was chosen based on our previous studies (Arenberg et al., J. Exp. Med. 184:981) and the *in vitro* biological activity data provided by the manufacturer. Maximal chemotactic activity of SLC for total murine T cells was found to be 100 ng/ml. For *in vivo* evaluation of SLC-mediated antitumor properties we used 5-fold more than this amount for each injection. At 4 months, mice were sacrificed, and lungs were isolated for quantification of tumor surface area. Tumor burden was assessed by microscopic examination of H&E-stained sections with a calibrated graticule (a 1-cm² grid subdivided into 100 1-mm² squares). A grid square with tumor occupying $>50\%$ of its area was scored as positive, and the total number of positive squares was determined as described previously (Sharma et al., J. Immunol., 163: 5020–5028, 1999). Ten separate fields from four histological sections of the lungs were examined under high-power (X 20 objective). Ten mice from each group were not sacrificed so that survival could be assessed.

4. Cytokine Determination from Tumor Nodules, Lymph Nodes, and Spleens.

The cytokine profiles in tumors, lymph nodes, and spleens were determined in both SLC and diluent-treated mice as described previously (Sharma et al., J. Immunol., 163: 5020–5028, 1999). Non-necrotic tumors were harvested and cut into small pieces and passed through a sieve (~~Bello~~ BELLCO, Vineland, NJ). Axillary lymph nodes and spleens were harvested from SLC-treated tumor-bearing, control tumor-bearing, and normal control mice. Lymph nodes and spleens were teased apart, RBC depleted with ddH₂O, and brought to tonicity with 1 x PBS. After a 24-h culture period, tumor nodule supernatants were evaluated for the production of IL-10, IL-12, GM-CSF, IFN- γ , TGF- β , VEGF, MIG, and IP-10 by ELISA and PGE-2 by EIA. Tumor-derived cytokine and PGE-2 concentrations were corrected for total protein by Bradford assay (~~Sigma-Chemical~~ SIGMA CHEMICAL Co.). For cytokine determinations after secondary stimulation with irradiated tumor cells, splenocytes (5×10^6 cells/ml), were cocultured with irradiated (100 Gy, Cs¹³⁷ x-rays) CC-10 TAg tumor cells (10^5 cells/ml) at a ratio of 50:1 in a total volume of 5ml. After a 24-h culture, supernatants were harvested and GM-CSF, IFN- γ , and IL-10 determined by ELISA.

5. Cytokine ELISA.

Cytokine protein concentrations from tumor nodules, lymph nodes, and spleens were determined by ELISA as described previously (Sharma et al., *Gene Ther.*, **4**: 1361–1370, 1997). Briefly, 96-well ~~Costar~~ COSTAR (Cambridge, MA) plates were coated overnight with 4 µg/ml of the appropriate antimouse mAb to the cytokine being measured. The wells of the plate were blocked with 10% FBS (~~Gemini-Bioproducts~~ GEMINI BIOPRODUCTS) in PBS for 30 min. The plate was then incubated with the antigen for 1 h, and excess antigen was washed off with PBS/Tween 20. The plate was incubated with 2 µg/ml of biotinylated mAb to the appropriate cytokine (~~PharMingen~~ PHARMINGEN) for 30 min, and excess antibody was washed off with PBS/Tween 20. The plates were incubated with avidin peroxidase, and after incubation in O-phenylenediamine substrate to the desired extinction, the subsequent change in color was read at 490 nm with a ~~Molecular Devices~~ MOLECULAR DEVICES Microplate Reader (Sunnyvale, CA). The recombinant cytokines used as standards in the assay were obtained from ~~PharMingen~~ PHARMINGEN. IL-12 (~~Biosource~~ BIOSOURCE) and VEGF (~~Oncogene—Research—Products~~ ONCOGENE RESEARCH PRODUCTS, Cambridge, MA) were determined using kits according to the manufacturer's instructions. MIG and IP-10 were quantified using a modification of a double ligand method as described previously (Standiford et al., *J. Clin. Investig.*, **86**: 1945–1953, 1990). The MIG and IP-10 antibodies and protein were obtained from R&D (Minneapolis, MN). The sensitivities of the IL-10, GM-CSF, IFN-γ, TGF-β, MIG, and IP-10 ELISA were 15 pg/ml. For IL-12 and VEGF the ELISA sensitivities were 5 pg/ml.

5. PGE2 EIA.

PGE2 concentrations were determined using a kit from ~~Cayman-Chemical~~ CAYMAN CHEMICAL Co. (Ann Arbor, MI) according to the manufacturer's instructions as described previously (Huang et al., *Cancer Res.*, **58**: 1208–1216, 1998). The EIA plates were read by a ~~Molecular Devices~~ MOLECULAR DEVICES Microplate reader (Sunnyvale, CA).

6. Flow Cytometry.

For flow cytometric experiments, two or three fluorochromes (PE, FITC, and Tri-color; ~~PharMingen~~ PHARMINGEN) were used to gate on the CD3T-lymphocyte population of tumor nodule, lymph node, and splenic single cell suspensions. DCs were defined as the CD11c and DEC 205 bright populations within tumor nodules, lymph nodes, and spleens. Cells were identified as lymphocytes or DCs by gating based on forward and side scatter profiles. Flow cytometric analyses were performed on a FACScan flow cytometer (~~Becton-Dickinson~~ BECTON DICKINSON, San Jose, CA) in the University of California, Los Angeles, Jonsson Cancer Center Flow Cytometry Core Facility. Between 5,000 and 15,000 gated events were collected and analyzed using Cell Quest software (~~Becton-Dickinson~~ BECTON DICKINSON).

7. Intracellular Cytokine Analysis.

T lymphocytes from single cell suspensions of tumor nodules, lymph nodes, and spleens of SLC-treated and diluent treated CC-10 TAg transgenic mice were depleted of RBC with distilled, deionized H₂O and were evaluated for the presence of intracytoplasmic GM-CSF and IFN- γ . Cell suspensions were treated with the protein transport inhibitor kit Golgi Plug (~~PharMingen~~ PHARMINGEN) according to the manufacturer's instructions. Cells were harvested and washed twice in 2% FBS/PBS. Cells (5×10^5) were resuspended in 200 μ l of 2% FBS/PBS with 0.5 μ g of FITC-conjugated mAb specific for cell surface antigens CD3, CD4, and CD8 for 30 min at 4°C. After two washes in 2% FBS/PBS, cells were fixed, permeabilized, and washed using the Cytofix/Cytoperm kit (~~PharMingen~~ PHARMINGEN) following the manufacturer's protocol. The cell pellet was resuspended in 100 μ l of Perm/Wash solution and stained with 0.25 μ g of PE-conjugated anti-GM-CSF and anti-IFN- γ mAb for intracellular staining. Cells were incubated at room temperature in the dark for 30 min and washed twice, resuspended in 300 μ l of PBS/2% paraformaldehyde solution, and analyzed by flow cytometry.

Please amend the paragraphs at page 75, line 20 to page 76, line 20 as follows:

A weakly immunogenic lung cancer, Lewis lung carcinoma (3 LL, H-2^b) was utilized for assessment of cytokines important for SLC-mediated anti-tumor responses in vivo. The cells were routinely

cultured as monolayers in 25 cm³ tissue culture flasks containing RPMI 1640 medium (~~Irvine Scientific~~ IRVINE SCIENTIFIC, Santa Anna, CA) supplemented with 10% fetal bovine serum (FBS) (~~Gemini Bioproducts~~ GEMINI BIOPRODUCTS, Calabasas, CA), penicillin (100 U/ml), streptomycin (0.1 mg/ml), 2mM glutamine (~~JRH Biosciences~~ JRH BIOSCIENCES, Lenexa, KS) and maintained at 37^o C in humidified atmosphere containing 5% CO₂ in air. The cell lines were mycoplasma free and cells were utilized up to the tenth passage before thawing frozen stock cells from liquid N₂. For tumorigenesis experiments, 10⁵ 3LL tumor cells were inoculated by s.c. injection in the right supra scapular area of C57Bl/6 and tumor volume was monitored 3 times per week. Five day established tumors were treated with intratumoral injection of 0.5 µg of murine recombinant SLC or PBS diluent (~~Peppo Tech~~ PEPRO TECH, Rocky Hill, NJ) administered three times per week for two weeks. The endotoxin level reported by the manufacturer was less than 0.1ng per µg (1EU/µg) of SLC. The amount of SLC (0.5µg) used for injection was determined by the *in vitro* biological activity data provided by the manufacturer. Maximal chemotactic activity of SLC for total murine T cells was found to be 100 ng/ml. For in vivo evaluation of SLC-mediated anti-tumor properties we utilized 5 fold more than this amount for each intratumoral injection. Tumorigenesis experiments were also performed in which equivalent amounts of murine serum albumin were utilized (~~Sigma~~ SIGMA, St. Louis, Mo) as an irrelevant protein for control injections. 24 hours prior to SLC treatment, and then three times a week, mice were treated i.p. with 35 mg/dose of anti-IP-10 or anti-MIG, and 100µg/dose of purified IFN-γ (ATCC R4562) or 35mg/dose of control antibody for the duration of the experiment. At doses of antibody administered there was a significant in vivo depletion of the respective cytokines at the tumor site. Two bisecting diameters of each tumor were measured with calipers. The volume was calculated using the formula (0.4) (ab²), with “a” as the larger diameter and “b” as the smaller diameter.

Cytokine ELISA

MIG, IP-10 and IFN-γ were quantified using a modification of a double ligand method as previously described. The MIG and IP10 antibodies and recombinant cytokine proteins were from R&D (Minneapolis, MN). The IFN-γ antibodies pairs and recombinant cytokine were from ~~PharMingen~~ PHARMINGEN. The sensitivities of the IFNγ, MIG and IP-10 ELISA were 15 pg/ml.